

ORIGINAL RESEARCH

Treatment of Circadian Disorder with Premature Ovarian Insufficiency With the Nourishing Yin and Tonifying Yang Sequential Method with Femoston

Qianwen Ma, PhD; Xiangdi Ju; Yong Tan, PhD

ABSTRACT

Objective • The clinical effects of the nourishing Yin and tonifying Yang sequential method with Femoston was explored in treating circadian disorder with premature ovarian insufficiency (POI).

Method • We enrolled 600 patients with circadian disorder and POI in a prospective study and divided the patients into 2 groups: an experimental and a control group. Both groups were treated with Femoston and the experimental group also received nourishing Yin and tonifying Yang sequential method. We observed the overall response rate, Kupperman Index, number of adverse events, and the levels of prostaglandin E₂ (E₂), follicle-stimulating hormone (FSH), luteinizing hormone (LH), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), as well as peak systolic

velocity (PSV), pulsatility index (PI), resistance index (RI), maximum ovarian diameter (MOD) and antral follicle count (AFC).

Results • The experimental group also exhibited elevated TC, TG, LDL-C, MOD and AFC after treatment, whereas the control group did not. Compared with the control group, the experimental group had a higher overall response rate, E₂, FSH, LH, HDL-C, PSV, MOD, AFC, a lower Kupperman Index, TC, TG, LDL-C, PI, RI and number of adverse events.

Conclusions • In patients with circadian disorder with POI, the nourishing Yin and tonifying Yang sequential method with Femoston improved ovarian function, blood supply to the ovaries and sex hormone levels and lowered blood lipids with acceptable safety parameters. (*Altern Ther Health Med.* 2022;28(4):50-54)

Qianwen Ma, PhD, Gynecology Department, Zhenjiang Hospital of TCM, Zhenjiang, China; TCM Department, Hangzhou Ninth People's Hospital, Hangzhou, China. **Xiangdi Ju**, Gynecology Department, Zhenjiang Hospital of TCM, Zhenjiang, China. **Yong Tan, PhD**, Reproductive Medicine Department, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, China.

Corresponding author: Xiangdi Ju,
E-mail: 339212428@qq.com

Corresponding author: Yong Tan, PhD
E-mail: nzyszy@163.com

INTRODUCTION

With the rapid development of society, people experience increased stress levels from both life and work. The percentage of people who stay up late, work late shifts and irregular daily schedules is increasing, leading to a higher risk for circadian disorders and an elevated incidence of gynecological disorders in females such as spontaneous abortion, menstrual disorders,

infertility and premature ovarian insufficiency (POI),¹⁻⁴ which refers to ovarian hypofunction before age 40 years. The main clinical manifestations are irregular or a lack of menstrual cycles and long-term infertility. The occurrence of POI is closely related to age, genetics, environment, immunity and history of surgery. Indicators that reflect POI include levels of follicular stimulating hormone (FSH), luteinizing hormone (LH) and anti-Müllerian hormone (AMH), antral follicle count (AFC) and the number of growing follicles and oocytes after ovarian stimulation. As a disease that has a serious impact on female fertility, POI increases medical costs and physical and mental burdens and reduces quality of life (QoL).⁵⁻⁹ Currently, hormone replacement therapies (HRT) such as Femoston (oestradiol/oestradiol dydrogesterone) are common treatments in Western medicine for POI. However, the long-term effects of HRT are not satisfactory.¹⁰

The nourishing Yin and tonifying Yang sequential method was proposed by Professor Yong Tan¹¹ based on the “interactions between man and universe” in traditional Chinese medicine (TCM), which emphasizes the core notion of the “waxing and waning of Yin and Yang” during the female reproductive cycle. It stems from the theories of Professor Guicheng Xia¹², who

Table 1. Comparison of Baseline Data in the Two Groups

Item	n	Experimental group	Control group	χ^2/t	P value
Age(y)	600	31.92 ± 4.89	32.07 ± 5.62	0.36	.72
Disease course	600	12.19 ± 4.99	12.77 ± 4.81	1.44	.15
BMI (kg/cm ²)	600	22.18 ± 2.22	22.29 ± 2.45	0.61	.54
Secondary amenorrhea	186	95	91	0.12	.72
Oligomenorrhea	172	84	88	0.13	.72
Functional uterine bleeding	129	65	64	0.01	.92
Primary infertility	193	98	95	0.07	.79
Secondary infertility	308	152	156	0.11	.74
Menstrual extension	106	52	54	0.05	.83
T (ng/dl)	600	48.11 ± 5.18	47.93 ± 4.88	0.44	.66
LH (mIU/ml)	600	20.62 ± 7.99	21.18 ± 8.12	0.85	.40
FSH (mIU/ml)	600	37.33 ± 8.21	36.55 ± 8.78	1.12	.26
E ₂ (pg/ml)	600	20.71 ± 7.99	21.12 ± 5.44	0.92	.36
PRL(ng/ml)	600	14.71 ± 3.09	14.61 ± 3.12	0.42	.67
AMH (ng/ml)	600	0.93 ± 0.66	1.03 ± 0.87	1.58	.11
PSV (cm/s)	600	9.90 ± 1.81	9.63 ± 1.83	1.81	.07
PI	600	1.87 ± 0.61	1.82 ± 0.60	1.13	.26
RI	600	1.18 ± 0.50	1.22 ± 0.53	0.90	.37
Kupperman score	600	27.33 ± 4.37	27.61 ± 4.69	0.76	.45
TG (mmol/l)	600	2.38 ± 0.61	2.39 ± 0.55	0.21	.83
TC (mmol/l)	600	4.73 ± 0.44	4.75 ± 0.56	0.63	.53
LDL-C(mmol/l)	600	3.89 ± 0.36	3.94 ± 0.36	1.65	.10
HDL-C(mmol/l)	600	1.11 ± 0.45	1.15 ± 0.49	1.08	.28
AFC	600	0.72 ± 0.65	0.74 ± 0.72	0.36	.72
MOD (mm)	600	16.38 ± 2.53	16.39 ± 2.65	0.04	.97

Abbreviations: AFC, antral follicle count; AMH, anti-Müllerian hormone; BMI, body mass index; FSH, follicle-stimulating hormone; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; LH, luteinizing hormone; MOD, maximum ovarian diameter; PI, pulsatility index; PRL, prolactin; PSV, peak systolic velocity; RI, resistance index; T, testosterone; TC, total cholesterol; TG, triglycerides.

described the regulation of the menstrual cycle rhythm, which simplifies the menstrual cycle by changing the classification of the fourth, fifth, and seventh stage to only premenstrual and postmenstrual. TCM treats patients with POI, infertility and other reproductive endocrine diseases by sequentially giving nourishing Yin and tonifying Yang, consistent with the mechanisms of kidney-reinforcing and menstrual cycle-regulating therapy, which have shown satisfactory clinical effectiveness. Professor Tan's team found that the nourishing Yin and tonifying Yang sequential method promoted follicular development, improved the ovarian micro-environment and function and endometrial receptivity, increased the level of stem cell factor in follicular fluid and thickness of the endometrium and decreased the occurrence of ovarian hyperstimulation syndrome, thus enhancing fertility and the rate of high-quality embryos and pregnancy in patients with reproductive endocrine dysfunction.

Our study prospectively analyzed ovarian stimulation in 600 patients with circadian disorder and POI and explored

the effectiveness of the nourishing Yin and tonifying Yang sequential method plus Femoston.

METHODS

Study Participants

We enrolled 600 outpatients from September 2016 to December 2019 from the department of reproductive medicine of Jiangsu Province Hospital of Chinese Medicine, China, who were diagnosed with circadian disorder combined with POI. We employed the following diagnostic criteria for POI set by the European Society for Human Reproduction and Embryology (ESHRE): females (1) younger than 40 years old; (2) with menopause or oligomenorrhea for longer than 4 months; and (3) with follicle-stimulating hormone (FSH) levels higher than 25 U/L 2 consecutive times over 4 weeks.

Inclusion criteria

Patients who were (1) younger than age 40 years, (2) had a body mass index (BMI) of 18 to 28 kg/m² and (3) had a diagnosis of circadian disorder and POI.

Exclusion criteria

Patients were excluded if they (1) exhibited physiological and genetic defects; (2) had disease of the reproductive system, endocrine system, heart, liver, kidney, brain or blood; (3) had mental illness; or (4) had an allergy to components of the medication. Furthermore, patients who were using hormonal drugs or participating in other drug clinical trials were excluded from the study.

Treatment

Patients in the experimental group (n = 300) were treated with nourishing Yin and tonifying Yang plus Femoston, while the control group (n = 300) received Femoston therapy alone. Baseline characteristics of the

patients in the 2 groups were comparable (see Table 1). All study participants signed an informed consent form and the study procedures were approved by the institute's ethics committee.

Treatment Method

In the experimental group, patients were treated with 1 mg oral estradiol (E₂) per day from the 1st to the 14th day of menstruation and 10 mg E₂ dydrogesterone per day from the 15th to the 28th day of menstruation for 3 menstrual cycles. The Yin-nourishing prescription was administered in conjunction with E₂ and consisted of 12g Chinese angelica, 12g radix paeoniae alba, 10g adhesive rehmannia dried root, 10g dogwood, 12g semen cuscatae and 10g placenta hominis. While taking dydrogesterone, patients also received the Yang-replenishing prescription, which consisted of 15g radix dipsaci, 12g Chinese yam, 12g morinda officinalis, 10g herba epimedii, 10g fructus psoraleae, and 10g codonopsis pilosula. The prescriptions were adjusted according to the syndromes being treated, and the patients took 1 decoction per day. In brief, the herbs were soaked

in 500 ml water for 20 minutes and boiled for 20 minutes. The liquid was separated and the remaining herbs were boiled for 20 more minutes with 200 ml water. The liquid was consumed in the morning and evening. The Yin-nourishing prescription was administered for 7 days while the Yang-replenishing prescription was used for 14 days over 3 menstrual cycles, but was suspended during menses. Patients in the control group only received hormone therapy with Femoston. The drug administration mode is shown in Figure 1.

Outcome Measures

We documented the overall response rate, Kupperman Index and the levels of E₂, FSH, LH, TC, TGs, LDL-C and HDL-C, as well as PSV, PI, RI, MOD, AFC and the number of adverse events.

Statistical Analysis

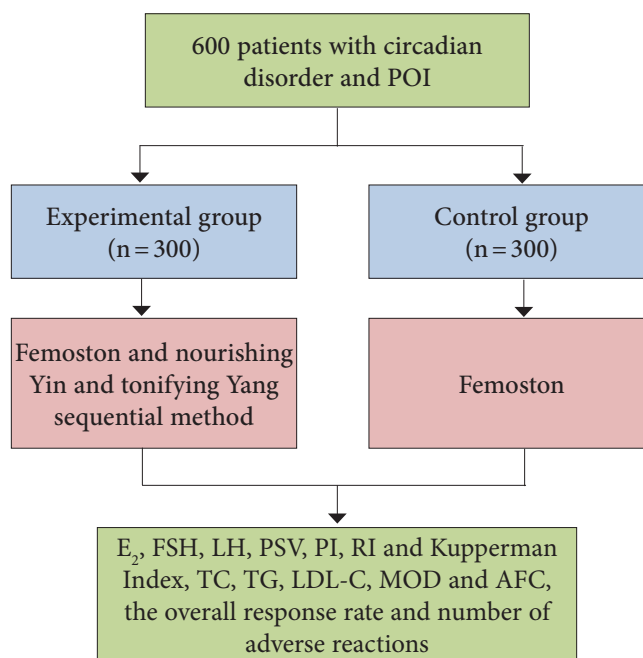
The mean values in the groups were compared by Student's *t* test and count data was tested by χ^2 test. *P* < .05 was considered statistically significant.

RESULTS

Before and After Treatment Comparison in Both Groups

The levels of E₂, FSH, LH, PSV, PI, RI and Kupperman Index in the 2 groups after treatment were higher than before treatment (*P* < .05). Patients in the experimental group after treatment also had higher TC, TG, LDL-C, MOD and AFC levels than before treatment (*P* < .05), while there was no difference between the 2 groups.

Figure 1. Drug administration mode.



Abbreviations: AFC, antral follicle count; E₂, estradiol; FSH, follicle-stimulating hormone; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; LH, luteinizing hormone; MOD, maximum ovarian diameter; PI, pulsatility index; PSV, peak systolic velocity; RI, resistance index; T, testosterone; TC, total cholesterol; TG, triglycerides.

Table 2. Comparison of Observation Indicators in the Two Groups Before and After Treatment

Item	Experimental Group					Control Group				
	n	Before treatment	After treatment	t	P value	n	Before treatment	After treatment	t	P value
Kupperman Index	300	27.33 ± 4.37	10.69 ± 2.59	22.09	<.0001	300	27.61 ± 4.69	19.64 ± 2.04	27.46	<.0001
E ₂ (pg/ml)	300	20.71 ± 7.99	71.38 ± 9.25	75.96	<.0001	300	21.13 ± 5.44	53.28 ± 6.91	65.68	<.0001
FSH (mIU/ml)	300	37.33 ± 8.21	14.69 ± 3.34	44.55	<.0001	300	36.55 ± 8.78	20.99 ± 5.08	27.01	<.0001
LH (mIU/ml)	300	20.62 ± 7.99	9.51 ± 1.75	23.27	<.0001	300	21.18 ± 8.12	14.81 ± 3.09	12.70	<.0001
TC (mmol/l)	300	4.73 ± 0.44	3.79 ± 0.43	26.53	<.0001	300	4.75 ± 0.56	4.68 ± 0.45	6.73	.08
TG (mmol/l)	300	2.39 ± 0.61	1.82 ± 0.60	11.31	<.0001	300	2.40 ± 0.55	2.32 ± 0.54	1.71	.09
LDL-C (mmol/l)	300	3.89 ± 0.36	2.32 ± 0.54	42.64	<.0001	300	3.94 ± 0.36	3.93 ± 0.34	1.79	.07
HCL-C (mmol/l)	300	1.11 ± 0.45	1.82 ± 0.60	15.93	<.0001	300	1.15 ± 0.09	12.3 ± 0.53	1.75	.08
PSV (cm/s)	300	9.90 ± 1.81	16.50 ± 2.63	36.97	<.0001	300	9.63 ± 1.83	10.82 ± 2.91	5.51	<.0001
RI	300	1.18 ± 0.50	0.96 ± 0.32	6.59	<.0001	300	1.22 ± 0.53	1.09 ± 0.44	3.02	.0028
PI	300	1.17 ± 0.51	1.17 ± 0.51	15.21	<.0001	300	1.82 ± 0.60	1.43 ± 0.68	7.70	<.0001
MOD (mm)	300	16.38 ± 2.53	19.01 ± 1.12	16.43	<.0001	300	16.39 ± 2.65	17.63 ± 1.13	7.64	<.0001
AFC	300	0.72 ± 0.65	4.30 ± 1.42	39.96	<.0001	300	0.74 ± 0.72	2.98 ± 0.19	29.60	<.0001

Abbreviations: AFC, antral follicle count; E₂, estradiol; FSH, follicle-stimulating hormone; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; LH, luteinizing hormone; MOD, maximum ovarian diameter; PI, pulsatility index; PRL, prolactin; PSV, peak systolic velocity; RI, resistance index; T, testosterone; TC, total cholesterol; TG, triglycerides.

Table 3. Comparison of Observation Indicators in the Two Groups After Treatment

Item	N	Experimental Group	Control Group	χ^2/t	P value
Overall response rate	600	88%	71%	17.93	<.0001
Kupperman Index	600	10.96 ± 2.59	19.64 ± 2.04	45.63	<.0001
E ₂ (pg/ml)	600	71.38 ± 9.25	53.28 ± 6.91	27.13	<.0001
FSH (mIU/ml)	600	14.69 ± 3.34	20.99 ± 5.08	17.93	<.0001
LH (mIU/ml)	600	9.51 ± 1.75	14.81 ± 3.09	25.88	<.0001
TC (mmol/l)	600	3.79 ± 0.43	4.68 ± 0.45	24.88	<.0001
TG (mmol/l)	600	1.82 ± 0.60	1.23 ± 0.53	10.69	<.0001
LDL-C (mmol/l)	600	2.32 ± 0.54	3.93 ± 0.34	43.70	<.0001
HDL-C (mmol/l)	600	1.82 ± 0.60	1.23 ± 0.53	12.70	<.0001
PSV (cm/s)	600	16.50 ± 2.63	10.82 ± 2.91	25.08	<.0001
PI	600	0.96 ± 0.32	1.09 ± 0.44	4.28	<.0001
RI	600	1.17 ± 0.51	1.43 ± 0.68	5.23	<.0001
MOD (mm)	600	19.01 ± 1.12	17.63 ± 1.13	15.05	<.0001
AFC	600	4.30 ± 1.42	2.98 ± 1.09	12.72	<.0001
No. of Adverse events	600	5	28	16.96	<.0001

Abbreviations: AFC, antral follicle count; E₂, estradiol; FSH, follicle-stimulating hormone; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; LH, luteinizing hormone; MOD, maximum ovarian diameter; PI, pulsatility index; PSV, peak systolic velocity; RI, resistance index; TC, total cholesterol; TG, triglycerides.

Comparison Between the Two Groups After Treatment

Compared with the control group, the experimental group had a higher overall response rate, E₂, HDL-C, PSV, MOD and AFC after treatment than the control group (*P* < .05). In addition, the experimental group showed a lower Kupperman index, FSH, LH, TC, TG, LDL-C, PI, RI and number of adverse events than the control group (*P* < .05). The specific adverse events were nausea, vomiting, loss of appetite, etc. Therefore, the experimental group was better than the control group regarding the overall response rate, E₂, HDL-C, PSV, MOD, AFC, Kupperman index, FSH, LH, TC, TG, LDL-C, PI, RI and safety.

DISCUSSION

Circadian disorder may cause various endocrine conditions including diseases of the reproductive system. The number of patients diagnosed with ovulation failure induced by circadian disorder has increased recently, which may be attributable to work shifts, staying up late and irregular daily schedules, making it impossible or difficult for patients to return to a normal rhythm. These disturbances can lead to POI, polycystic ovarian syndrome and infertility in women.¹³ Many doctors believe that the etiology and pathogenesis of POI are due to a deficiency of Yin and Yang in the kidney. The consequences are delayed menstruation, little menstruation, amenorrhea, infertility and so on. In this study, we examined the treatment of circadian disorder combined with POI with the nourishing Yin and tonifying Yang sequential method plus Femoston.

The levels of E₂, FSH, LH, PSV, PI, RI, and Kupperman Index in the 2 groups after treatment were higher than before treatment. Patients in the experimental group also had higher levels of TC, TG, LDL-C, MOD, and AFC after treatment than before treatment, while patients in the control group did not. Compared with the control group, the experimental group had a higher overall response rate, E₂, HDL-C, PSV, MOD and AFC after treatment than the control group. In addition, the experimental group showed a lower Kupperman Index, FSH, LH, TC, TG, LDL-C, PI, RI and number of adverse events than the control group. Therefore, treatment with the nourishing Yin and tonifying Yang sequential method plus Femoston was better than Femoston alone with regard to the overall response rate, E₂, HDL-C, PSV, MOD, AFC, Kupperman Index, FSH, LH, TC, TG, LDL-C, PI, RI and had a better safety profile.

Early animal experiments indicate that rats with circadian disorder have an abnormal gonadal axis, endocrine disorders, an out-of-rhythm estrus cycle, prolonged estrus period and ovulation failure. The nourishing Yin and tonifying Yang sequential method promotes follicular development and enhances luteal function by improving the hormonal metabolism and synthesis of granular and theca cells.¹⁴⁻¹⁸ Our clinical data demonstrate that this treatment applies to patients with circadian disorder combined with POI. Our clinical data indicated that the nourishing Yin and tonifying Yang sequential method plus Femoston was more effective and safer than Femoston alone.

The nourishing Yin and tonifying Yang sequential method was proposed by Professor Yong Tan (2004)¹¹ with the theory of regulating the rhythm of the menstrual cycle, which nourishes Yin postmenstruum and replenishes Yang premenstruum. The state after menses is important for the foundation of a new cycle. Therefore, the treatment is focused on herbs that nourish Yin and supplement the blood, like Chinese angelica, dogwood, adhesive rehmannia dried root and radix paeoniae alba. Just before the menses is a period when Yang Qi migrates, and therefore the main aim of premenstrual treatment is the reinforcement of the kidney to strengthen Yang using herbs such as morinda officinalis, radix dipsaci, fructus psoraleae, and herba epimedii. Thus, nourishing Yin and tonifying Yang may enhance ovarian function by promoting follicular development after menses and luteal function before menses. Femoston regulates the menstrual cycle and improves sex hormone levels, but has serious long-term effects on AFC and MOD. Nevertheless, the combination of TCM with Western medicine can be complementary.¹⁹⁻²⁰

CONCLUSION

In summary, our study demonstrated that the nourishing Yin and tonifying Yang sequential method plus Femoston improved ovarian function and blood supply, elevated sex hormone levels and decreased blood lipids in patients with circadian disorder combined with POI with acceptable safety parameters.

CONFLICT OF INTEREST

None.

FUNDING

This research was supported by the Natural Science Foundation of China (No. 81873333) and Zhenjiang Social Development of Key Research Project (No.SH2021025).

REFERENCES

1. Itani O, Kaneita Y. The association between shift work and health: a review. *Sleep Biol Rhythms*. 2016;14(3):231-239.
2. Dumont M, Paquet J. Progressive decrease of melatonin production over consecutive days of simulated night work. *Chronobiol Int*. 2014;31(10):1231-1238.
3. Bonmaticarion Ma, Arguellesprieto R, Martinezmadrid MJ, et al. Protecting the melatonin rhythm through circadian healthy light exposure. *J Neurophysiol*. 2014;15(12):23448-23500.
4. Dibner C, Gachon F. Circadian dysfunction and obesity: is leptin the missing link? *Cell Metab*. 2015;22(3):359-360.
5. Daniela AY, Patricia MS. Influence of sexual function on the social relations and quality of life of women with premature ovarian insufficiency. *Rev Bras Ginecol Obstet*. 2018;40:66-71.
6. Boxian H, Jiafeng L, Chenyue D, et al. Exosomes derived from human adipose mesenchymal stem cells improve ovary function of premature ovarian insufficiency by targeting SMAD. *Stem Cell Res Ther*. 2018;9(1):216-227.
7. Webber L, Davies M, Anderson R, et al. ESHRE Guideline: management of women with premature ovarian insufficiency. *Hum Reprod*. 2016;31(5):926-937.
8. Agnieszka PS, Adam C, Monika G, et al. Premature ovarian insufficiency: the context of long-term effects. *J Endocrinol Invest*. 2016;39:983-990.
9. Saioa T, Pinar K, Lubna P. Premature ovarian insufficiency—an update on recent advances in understanding and management. *F1000 Res*. 2017;6:2069.
10. Jing L, Jun Z, Fubo T, et al. Effects of Ziyin Jianghuo Ningxin decoction plus dehydroepiandrosterone and femoston in treatment of patients with menopausal symptoms. *J Trad Chinese Med*. 2018;38(5):787-796.
11. Qingling Ren, Yong Tan. Intervention of sequential method of nourishing yin and tonifying yang on serum IGF-1 and sex hormone in patients with PCOS[J]. *Jiangsu Journal of Traditional Chinese Medicine*. 2006;27(5):28-30.
12. Bo Xu, Yanyun Yin, Yong Tan. Experience of Xia Guicheng, a master of Chinese medicine, in the treatment of anovulatory infertility[J]. *China Journal of Traditional Chinese Medicine and Pharmacy*. 2017;32(7):2983-2985.
13. Brum MCB, Filho FFD, Schnorr CC, et al. Shift work and its association with metabolic disorders. *Diabetol Metab Syndr*. 2015;7(1):1-7.
14. Yujie Zhang, Qianwen Ma, Yong Tan. Study of effect of continuous illumination on reproductive endocrine of female rats and intervention effect of the sequential therapy of nourishing Yin and tonifying Yang. *J Nanjing University Chinese Med*. 2017;33(4):386-390.
15. Yijie Zou, Yong Tan, Xingli Tong. Effect of nourishing Yin and tonifying Yang traditional Chinese medicine on ovarian related factors in photodamaged rats. *Shi Zhen Chinese Med*. 2014(11):2590-2594.
16. Qianwen Ma, Yong Tan. Clinical observation of the sequential method of nourishing Yin and tonifying Yang combined with CC/HMG/HCG in the treatment of daily rhythm disorder and ovulation disordered infertility. *J Sichuan University (Medical Edition)*. 2017, 48(4):635-636.
17. Qianwen Ma, Yong Tan. Study of effect of light pollution on rat ovarian MicroRNA and sequential method of nourishing Yin and tonifying Yang. *Modernizat Trad Chinese Med*. 2019;21(11):2499-2507.
18. Xu W, Zhou F, Li C, et al. Application of Femoston in hormone replacement treatment-frozen embryo transfer and its clinical outcomes. *J Chinese Med*. 2013;93(47):3766-3769.
19. Roberto A, Luvero D, Grazia A, et al. Hormone replacement therapy in cancer survivors: Utopia? *Crit Rev Oncol Hemat*. 2018;124:51-60.
20. Wang Y, Lewin N, Qaoud Y, et al. The oncologic impact of hormone replacement therapy in premenopausal breast cancer survivors: A systematic review. *Breast*. 2018;40:123-130.